Stefan Petrovic
California Institute of Technology
Linker proteins tie the Nuclear Pore together
Linker proteins tie the Nuclear Pore Complex (NPC) together

Cry
Stefan Petrovic
Hoelz lab, Caltech
April 12th-14th, 2021
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Daniel Mann
Forschungszentrum Juelich
Macromolecular organization of membrane-associated Atg18 oligomers
Macromolecular organization of membrane-associated Atg18 oligomers

DANIEL MANN
ATG18
A KEY PLAYER IN MACROAUTOPHagy

- Autophagy
  - Intracellular degradation process
    (recycling and removal of material)
  - Encapsulation, delivery to lysosome
- Atg18: formation and elongation of phagophore
- Atg18 binds PI3P and PI(3,5)P$_2$
- Atg18 assembles into helical tubes
ATG18 on cellular membranes
Tomography of atg18 assemblies

- PySeg package / Relion for subtomo averaging

Double membrane binding of a ~50 kDa protein

Martinez-Sanchez et al. Nat.Meth. 2020
Ernst Ruska Centre 3 @ Forschungszentrum Jülich

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www.sachse.fz-juelich.de

www.fz-juelich.de/er-c/er-c-3

https://erc-3-cloud.fz-juelich.de/FSC_Server/
Giulia Paris
University of Cambridge
Structural studies of the membrane-bound RNA degradosome of E.coli
Structural studies of the membrane-bound RNA degradosome of *E. coli*

RNA degradosome:

- Multi-enzyme complex involved in mRNA turnover and gene expression regulation
- Bound to the inner membrane of *E. coli*
- Flexible and natively unstructured scaffold domain

*Bandrya et al.*, Biochim Biophys Acta, 2013;
Structural studies of the membrane-bound RNA degradosome of *E. coli*

- Reconstitution of the complex on membranes
- Cryo-electron tomography
- Measure of the 1D-Radial Density Profile (1D-RDP)

Tom Dendooven

Dendooven, Paris et al., in preparation
Structural studies of the membrane-bound RNA degradosome of *E. coli*

Interaction with ribosomes: cryo-electron tomography and subtomogram averaging

Averaged ribosomes mapped back in original tomogram

30S ribosome map – 16 iterations; 9938 particles: 11.7 Å
Nadia Herrera
University of California, San Francisco
Investigation of the ultrastructural dynamics of the mycobacterial ESX-1 complex
Investigating the ultrastructure of mycobacterial ESX-1 secretion systems

Nadia Herrera, PhD

NCCAT Tomography Short Course
April 12-14, 2021
**ESX-1** has been assumed follow paradigm of secretion systems

Adapted from:
ESX-1 foci in \textit{M. smegmatis} correlate to a large oligomer

Correlates to \(~100\)MDa complex, \(~50\) ESX-1 complexes.

ESX-1 assembly in whole cells?

PDBID: 6UMM
Shannon Kordus
Vanderbilt University Medical Center
Understanding toxin secretion in Clostridioides difficile
How does a large protein get through the cell wall?
The *Clostridiodes difficile* toxins do not require a secretion tag or cell lysis for secretion.
Cryo-ET gives insights into the structure of the *C. difficile* cell wall during toxin secretion
Emily Armbruster
CUNY Advanced Science Research Center
Interactions and Structure of Ryanodine Receptor Clustering on the Sarcoplasmic Reticulum of Cardiac Muscle
Interactions and Structure of Ryanodine Receptor (RyR) Clustering on the Sarcoplasmic Reticulum of Cardiac Muscle

Emily Armbruster, PhD
Postdoc, des George Lab

April 13, 2021
RyR cluster size as well the RyR2 arrangement are dynamic, modulated, and correlated to calcium leaks and heart failure.

Cardiac Muscle

- Calcium binding one RyR channel can induce multiple RyR channels to open causing calcium spike
- Calcium spike induces muscle contraction
- Clustering of RyRs on membrane impact calcium spike
- Clustering can be modulated by phosphorylation, small molecules, and small accessory proteins

Heart failure correlation
Control heart
Heart that has failed
Modulation by phosphorylation
Control heart slice
Phosphorylated heart slice

Figure from Katz AM. Physiology of the Heart. 3rd ed. 2001
Kolstad et al. 2018
Ashgari et al. 2020
Preliminary data of the cardiac SR observed by cryo-ET

Pig Heart
- Break up tissue
- Two centrifugation steps
- Extrude

Microsomes of Sarcoplasmic Reticulum membranes

Incubate lacy carbon grid with an RyR antibody

Incubate with microsomes

[Images of electron microscopy images with a scale bar of 1 nm]
Angela Kirykowicz
University of Cambridge
Structure and function of the Type I secretion system transport machine in its cellular context
What Determines Substrate Specificity?

Diversity of quaternary structures in response to stress


Enzyme substrates

Virulence factor secretion

Structure and function of the Type I Secretion System transport machine in its cellular context
Gabriela Condezo
Spanish National Center for Biotechnology
Towards 3D visualization of virus assembly in the cell
Towards 3D visualization of virus assembly in the cell

Adenovirus

Structure of purified viral particles
Marabini et al. 2021
Sci Adv. 7(14)

Assembly mechanism
Condezo and San Martín. 2017 PLoS Pathog 27

Gabriela Condezo
Spanish National Biotechnology Centre
Adenovirus Assembly

TsV-N1 Assembly

Condezo and San Martín. 2017 PLoS Pathog 27
Kelli Hvorecny
University of Washington
Divergent Actin and a Lamellipodium-Like Structure in Giardia
The Actin Cytoskeleton of Giardia lamblia

Kelli L Hvorecny

Kollman & Paredez Groups
How does *Giardia* form its lamellipodium-like structure?

Paredez et al. 2011 PNAS
How does *Giardia* form its lamellipodium-like structure?
Many thanks!

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Structure of SARS-CoV-2 RBD baring contagious mutation in complex with ACE2
Structure of SARS-CoV-2 RBD baring contagious mutation in complex with ACE2

Nadav Elad

Electron Microscopy Unit
Weizmann Institute

Wiese et al (2020) Molecules in pathogenesis
*In vitro* evolution of SARS-CoV-2 RBD

Jiří Zahradník, ..., Orly Dym, Nadav Elad, Gideon Schreiber. SARS-CoV-2 RBD *in vitro* evolution follows contagious mutation spread, yet generates an able infection inhibitor. [https://www.biorxiv.org/content/10.1101/2021.01.06.425392v3](https://www.biorxiv.org/content/10.1101/2021.01.06.425392v3)
Structure of mutated SARS-CoV-2 RBD – ACE2

Jiří Zahradník, ..., Orly Dym, Nadav Elad, Gideon Schreiber. SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor. https://www.biorxiv.org/content/10.1101/2021.01.06.425392v3
Mark Kreutzberger
University of Virginia
Visualizing compressional distortions of the supercoiled bacterial flagellar filament in a thin layer of ice
Visualizing compressional distortions of the supercoiled bacterial flagellar filament in a thin layer of ice

Mark Kreutzberger
Egelman Lab
University of Virginia
Hong Zhan
University of Wisconsin-Madison
Deciphering the nano-machinery of RNA viral replication using Cryo-EM subtomogram averaging
Deciphering the Nano-machinery of RNA Viral Replication Using Cryo-EM Subtomogram Averaging

Host

Nuclei

Flask house virus (FHV)

Cryo-EM

Subtomogram Averaging

Hong Zhan
NCCAT Cryo-EM workshop
April 13th, 2021

Similar replication ultrastructure feature in RNA virus family

Coronavirus
Replication complex
RNA
Cytosol

Replication complex
Crown
RNA
Cytosol

Flock house virus (FHV)

FHV “Crown”

Mitochondria membrane
Replication factory membrane

Viral RNA

Ertel et al. eLife 2017

Unchwaniwala and Ahlquist. Science 2020
Wolff et al. Science 2020
Unchwaniwala, Zhan et al. PNAS 2020

4640 crowns from 59 tomograms
Unchwaniwala, Zhan et al. PNAS 2020

*: Membrane contact points

Hong Zhan
NCCAT Cryo-EM workshop
April 13th, 2021
Site-Specific Labeling of FHV Protein A C-Terminus

FHV genome

Replication process

FHV protein A C-terminus fused with GFP-11 tag

FHV protein A C-terminus fused with His tag for EM labeling

FHV protein A C-ter is accessible to the cytoplasm

Unchwaniwala, Zhan et al. PNAS 2020

Unchwaniwala, Zhan et al. PNAS 2020
FHV Protein A C-Terminus is located at Crown Apex

Unchwaniwala, Zhan et al. PNAS 2020

Replication/dsRNA synthesis model

Unchwaniwala, Zhan et al. PNAS 2020
Madhumati Sevvana
Purdue University
Towards high resolution structural virology for therapeutics development
Atomic resolution structural virology for therapeutics development

Madhu Sevvana
13th April 2021
NCCAT Tomography Course
Study antibody mediated neutralization of viruses for structure-based therapeutics and vaccine development

Why cryo-ET?
- Asymmetric complexes of icosahedral viruses and Abs
- 3D structure of pleomorphic viruses in complex with Abs
Jae Yang
University of Wisconsin-Madison
Studying in-situ viral infection with multi-modal cryogenic correlative FLM-FIB/SEM-ET and CorRelator
Exploring *in-situ* viral infection with cryogenic correlative FLM-FIB/SEM-ET and CorRelator

Jae Yang

Wright Laboratory & Midwest Center for Cryo-Electron Tomography (MCCET), Department of Biochemistry, University of Wisconsin-Madison

CorRelator supports:

- Cross-platform & system-independent correlations
- Accurate and flexible on-the-fly (with SerialEM) and post-acquisition correlation
- Combination of algorithmic and human interactive registration
- Intuitive, and user-friendly application

Plunge freezing -> cryo-FLM

Liquid ethane freezing

RT FLM -> Plunge freezing

Liquid ethane freezing

SerialEM-automated cryo-EM/ET data collection at ROI


Multi-modal correlative approaches to study respiratory syncytial virus infection in HeLa cells

HeLa cells infected by respiratory syncytial virus (red fluorescent signal), live-cell labeling of mitochondria and or microtubules (green fluorescent signal)
Cryo-ET of regions of interest (ROIs) in infected cells

Mitochondria (green) in infected (X) and uninfected, healthy. (X) HeLa cells

Tomogram slice (thickness of 30 nm) of ROIs in uninfected, healthy HeLa cells, Scale bar = 200 nm
Sagar Khavnekar
Max Planck Institute of Biochemistry
Algorithms, TEMs, and DualBeams... Making the most out of time left after coffee, beer, and whisky.
Algorithms, TEMs, and DualBeams... Making the most out of time left after coffee, beer, and whiskey.

Sagar Khavnekar
Max Planck Institute of Biochemistry
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